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TWO NEW SPECIES OF CHARACIUM.[1]

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(Plate 79.)

Characium gracilipes n. sp. Cellula 80–480 μ longa, 5–13 μ diam., regulariter curvata, parte media fusiformis, superne in setam longam, inferne in stipitem longam filiformem attenuata, rhizoideis minutissimis substrato affixa.

Cells 80–480 μ long, 5–13 μ diameter, regularly curved; middle part fusiform, tapering above into a long hair, below into a long filiform stipe, attached to the substratum by very minute rhizoids.— On the minute crustacean, *Branchipus vernalis*, Medford, Massachusetts, May, 1903, 1904, 1905, and 1906.

In May, 1903, while I was collecting with a class in biology, in Medford, Mass., on the edge of the Middlesex Fells, one of my students called attention to a small green object moving about in a small pool. On examination it proved to be a fairy shrimp, *Branchipus vernalis*, one of the animal forms for which I was searching, and as its color was so very unusual, a number of the animals were collected and carried to the laboratory. A microscopic examination showed that the green color was due to masses of green algae attached to various parts of the body, particularly to the appendages. As it seemed likely that the plant might be of interest and perhaps also might be desirable for distribution in the Phycotheca Boreali-Americana, some of the Branchipus were submitted to Mr. F. S. Collins for identification of the algae, who immediately reported that they were apparently two new species of Characium. Only a few of the

[1] Contributions from the Biological Laboratories of Tufts College, No. 48.

Branchipus were collected that spring; in the spring of 1904 a few more were collected from the same pond; but not until the spring of 1905, however, were conditions favorable for collecting the material in sufficient quantity for the P. B.–A. sets. At that time there was but little water in the pool, and it lowered so rapidly during the time Branchipus was present, that the animal was driven gradually toward deeper water. I was able to scoop up with a net from the muddy hole in the middle of the pool, about a pint of specimens, which I preserved in 5 % formaldehyde. Other pools in the vicinity were frequently but unsuccessfully examined. Only the one pool contained it, and in such abundance that a recent examination of the material collected in 1905 showed the Characia attached not only to Branchipus, but also to mosquito larvae, which had been overlooked at the time of collection. In May, 1906, a few sketches, not more than eight or ten, were made from a small number of specimens collected at that time, but the scarcity of Branchipus made it advisable to postpone further investigations until the next spring, when, it was hoped, material would be sufficiently abundant for a study of the reproduction. Failure to find the material in 1907 and in 1908 leads me to fear that it may have disappeared entirely; hence the publication of the present account founded almost wholly on material which was collected in 1905 and preserved in formalin, and from which sets for the P. B.–A. were prepared.[1]

Characium gracilipes occurs in greatest abundance on the flat surfaces of the two sides of the abdominal appendages of Branchipus, and rarely on the marginal hairs for which *Characium cylindricum* seems to show a preference. Both species, however, may be found on the head, antennae, and mouth-parts, and dorsal surface of body and tail. In respect to size, both *Characium gracilipes* and *Characium cylindricum* differ greatly from all species heretofore described: the smallest specimen of *Characium gracilipes* which I measured, 80 μ, was almost as long as the extreme length given by West[2] for *Characium ensiforme* Herm, 86 μ, which, he states, "is the most elongate species of the genus." The longest specimen of *Characium gracilipes*

[1] In order to prepare the material for the P.B.–A., about 500 formalin specimens of Branchipus were spread out to dry on a clean sheet of glass. When dry, they were fastened on mica with glue, two or three specimens on a piece. *Characium gracilipes* P. B.–A., No. 1270; *Characium cylindricum* P. B.–A., No. 1269.

[2] West, G. S. The British Freshwater Algae, Cambridge, 1904, p. 200.

which I observed, measured 480 μ, nearly six times as long as the maximum length for *Characium ensiforme* Herm.

The stipe and terminal hair are in line with the general curve of the body (fig. 4, pl. 79). The one cell stage of *Characium gracilipes* reminds one of *Closterium rostratum* Ehrenb., as figured by West.[1] In later stages during spore formation a more pronounced curvature frequently accompanies the elongation of the body, but it seldom exceeds an angle of 90°, and is always simple and in one direction.

The terminal hair, open throughout its entire length, but closed at its distal end, is about the same length in all specimens, and always shows a sharp demarcation from the fusiform body, from the apex of which it emerges. The lower end of the fusiform body tapers gradually into the stipe, also like the terminal hair, of capillary dimensions and hollow. The stipe is closed at a point very near its base. The base of this plant is remarkable in that it adds a new character for Characium, viz., the presence of minute rhizoids. These are of different forms, but the most typical is that shown in figures 3, 4, 5, 6 and 9 (pl. 79), which show two smooth, straight, slender, solid processes emerging from the closed base of the stipe, with which and with each other, the rhizoids form three approximately equal angles of about 120° respectively. This divergence is almost always evident, inasmuch as the rhizoids are in the same plane as the line of curvature, and as the specimens can naturally take but one position in the narrow space between the slide and cover. These processes, usually of the same length, vary from 2 to 10 μ. Fig. 22 (pl. 79) illustrates the largest observed.

Pl. 79, fig. 14, shows two rhizoids of approximately equal length, emerging at the same angle on one side of the stipe; fig. 15, rhizoid of one side with a hook turning inward; fig. 17, two rhizoids with hooks turning outward; fig. 16, two rhizoids depending from base, like a two-tined pitch-fork, not an uncommon condition; fig. 20, two rhizoids of the normal shape, but of unequal length; fig. 19, three rhizoids, a very rare condition; figs. 18 and 21, rhizoids of normal type with a tendency toward a branching which may vary from merely slight roughenings and thickenings to well-defined short branches. This condition is more common than that of fig. 19, but occurs less frequently than others. For a long time I really doubted this form, thinking the

[1] West, G. S. A monograph of the British Desmidiaceae. Vol. I, Pl. XXVI, fig. 1.

branched appearance might be due to the presence of detritus not unusual about the bases of the rhizoids. As efforts to dislodge these branch-like processes from the rhizoids failed, I concluded that in some cases, at least, the branching was real. The rhizoids penetrate the mucus on the surface of the appendages, and are well adapted to retain a hold on the swimming Branchipus. At first glance the rhizoids, particularly the short ones, might easily give the impression that the base consists of a disc seen in optical section, but careful observation with ordinary high powers, as well as with oil-immersion lens proves the fallacy of this supposition. This suggests a possibility that some of the structures, hitherto described in other species as discs, may have been, in some instances, rhizoids which, on account of insufficient magnification, have escaped detection.

The single parietal chromatophore shows a single conspicuous pyrenoid on the convex side of the cell, a position which the pyrenoids continue to occupy in the later stages during the entire process of transverse segmentation of the protoplast. The pyrenoid appears as a highly refractile body, spherical or ovoid in outline, separated from the chlorophyll-bearing part of the chromatophore by a narrow hyaline zone; always visible, it can be followed very easily in all the phases of its division and movement during the process of spore production. See figs. 3, 4, 5, 6 and 9 (pl. 79). The division of the pyrenoid is accompanied by a simultaneous division of the chromatophore into two equal parts. The pyrenoid, in dividing, first elongates, then by a median constriction, assumes a dumb-bell shape, and finally divides into two parts, each of which assumes the original rounded form. The division of the pyrenoid is the first visible evidence of spore development.

The usual method of spore formation is by repeated transverse division of the protoplast, which gives 32 cells arranged in a single series. Next, by longitudinal division, each of the 30 cells in the middle of the plant divides into two cells; at the same time, the basal and the terminal cells divide transversely, thus giving 64 cells, the maximum observed. See figs. 6 and 9 (pl. 79), the latter representing the basal (z) and terminal (x) portions of a plant containing 64 spores. The general plane of longitudinal division throughout the mother cell is usually continuous. Although this is the usual method of division, occasionally the longitudinal division occurs in an earlier stage. Fig. 5 (pl. 79) shows the longitudinal division appearing in the 4 cell stage. In other instances the longitudinal division first occurred in the 8 and in the 16 cell stage of the uniseriate type. The division of basal and terminal cells is always transverse, never longitudinal. At any stage, a count of cells and a careful examination of the pyrenoids leads to the conclusion that the divisions are approximately simultaneous throughout the length of the cell; the evidence, while not conclusive, suggests that a cell, once divided longitudinally does not divide again in any plane. Elongation of the individual is not always correlated with the progress of spore formation. Some individuals in incipient 2 or 4 cell stages are as long as others in fully developed 8 cell stages. Among the thousands of specimens that have been under observation, I have been unable to find any trace of the liberation of the spores, or of their germination; any empty cells; or any evidence of the spores assuming the rounded form characteristic of *Characium cylindricum* and of other species which have been hitherto described. A microscopic examination of Branchipus bearing the Characia always shows many specimens of *Characium gracilipes* broken, especially in the advanced stages of spore formation. These broken cells, however, present no evidence of the escape of the contents, save at the very ends, and there, only a few cells. There is no uniformity in the length of the broken pieces, nor in their stage of development. They may vary from early stages up to the 64 cell stage. Basal, middle, and distal portions all appear in the field at once, which leads one to think that the elongated *Characium gracilipes* had been broken up mechanically in the manipulation of the material under the cover-glass. An examination of entire specimens of Branchipus, under conditions where the chances of mechanical disturbance were at a minimum, still showed broken specimens. Moreover, inasmuch as the formalin had not caused any appreciable swelling, this broken condition could not be ascribed to the effect of the preservative. If fragmentation be the normal method of spore liberation, it is difficult to understand what becomes of the empty cells, and why there is no satisfactory evidence of liberation of the spores. If this fragmentation of *Characium gracilipes* be characteristic for the species, it may be that the liberated cells, on dissolution of the containing wall, assume the palmelloid state which has been described for other species. I have not seen evidence of the palmelloid condition. It seems strange that none of the germinating spores could be found; the smallest specimen observed measured 80 μ in length, and was fully developed in every respect.

Characium cylindricum n. sp. Cellula 24–430 μ longa, 10–20 μ diam., cylindrica, apice rotundata, basi in stipitem brevem attenuata; disco basali nullo.

Cell 24–430 μ long, 10–20 μ diam., cylindrical, with rounded apex, base tapering into a short stipe, without basal disc. — On the minute, crustacean, *Branchipus vernalis*, Medford, Massachusetts, May, 1903, 1904, 1905 and 1906.

Characium cylindricum, in the one cell stage (figs. 10, 11, 12 and 13, pl. 79), has a central nucleus and two parietal chromatophores. The chromatophores almost completely line the circumference of the cell, are slightly but distinctly separated from each other, thus affording a good view of the nucleus, situated near the middle of the cell and are without pyrenoids. Numerous small oil globules are often present in the cell. The lower portion of the cell tapers into a very short stipe with base rounded or pointed and often slightly bent at the place of attachment to the substratum.

Characium cylindricum occurs in greatest numbers upon the marginal hairs (on both main shaft and small branches) of the appendages of *Branchipus vernalis*, but may occur anywhere on the appendages.

Each individual is attached by means of a small, brownish, mucilaginous mass, distinct in outline, and usually very conspicuous on account of the detritus present upon its surface. See fig. 25 (pl. 79). The transparency of the chitinous wall of the hair affords an excellent opportunity to study the attachment from any point of view. In fig. 25 (pl. 79) the cell (a) is attached to the under side of the hair. The adhesive substance, when circular in outline, might easily be mistaken for a disc, did not its transparency permit a clear view of the pointed or rounded outline of the wall at the base of the cell. Whenever the cell is attached to two or more of the smaller hairs, they incline toward a common point of crossing, at which point the mucilaginous substance adheres. This method of attachment seems quite as well adapted to its function as do the rhizoids of *Characium gracilipes*. When *Characïum cylindricum* cells become detached from the hairs by mechanical disturbance of the cover glass, the adhesive substance usually sticks to the substratum, and the basal ends of the cells are clean, as illustrated in figs. 1, 2, 7, 8, 10, 11, 12, 13, and 24 (pl. 79). Observations under conditions of minimum mechanical disturbance show small patches of the adhesive substance here and there on the hairs, thus indicating that the cells of *Characium cylindricum* occasionally become detached from the live Branchipus.

By repeated transverse divisions the protoplast of the single cell divides into 8, 16, or 32 spores, fig. 24 (pl. 79). The regularity of approximately transverse divisions is frequently varied by a tendency toward the oblique, which may be confined to a few cells only, or may extend to the entire series. Longitudinal division usually begins after the protoplast has divided into 8 or 16 cells, though it may occur as early as the 4 cell stage. However, the above mentioned obliquity of the transverse divisions is usually so marked in the later stages (8–16 cell) that it is extremely difficult to determine whether division is transverse or longitudinal. In the individual illustrated in fig. 1 (pl. 79) there are eight spores, apparently motile, a condition still further confirmed not only by their arrangement and shape, but also by the presence of the hyaline papilla (p) at the anterior end, in connection with which I observed evidence of flagellae too indistinct to be studied. One of the spores (b) is seen escaping from a lateral aperture. There were but eight spores in the specimen shown in fig. 1 (pl. 79), but the unoccupied space in the containing cell would seem to indicate that the original number might have been 16, eight of which had been liberated. Fig. 7 (pl. 79) illustrates a cell containing 32 spores, which, from all appearances, were motile at the time of fixation. As the small amount of unoccupied space in the cell precludes 64 as the original number of the spores, it seems safe to assume that none of the spores had escaped. Apparently not more than eight or ten of the spores had been actively motile up to the time of fixation; moreover, with exception of the two detached spores near the base, the spores in the lower two thirds of the cell present the usual appearance of the protoplast after its division into 32 cells. The difficulty, previously mentioned, of interpreting the direction of planes of division is well illustrated in this specimen.

In another type of spore formation, by a process of segmentation, in its early stages indistinguishable from the foregoing, the protoplast divides into a very large number of small spores (fig. 8, pl. 79). In the more elongated specimens the number of these small spores certainly exceeds 1000, and in some cases perhaps 2000. Their number is not constant, however, as advancement in segmentation is not coordinated with the length of the specimen. Moreover, it is certain that these small spores, when fully developed and ready to escape from the cell, are not of uniform size. The few scattered spores near the base of the cell, the perforated apex of which appears in fig. 23 (pl. 79), measured

3 μ, certainly much larger than those which appear in fig. 8 (pl. 79). In the formalin material it was impossible to distinguish any details with respect to the flagellae of these spores, or to determine what becomes of any of the motile cells. Perhaps they are the micro- and macro-zoospores mentioned by Oltmanns,[1] as described by Reinhardt.[2]

Although *Characium cylindricum* is characterized by so many features, visible nucleus, two chromatophores, oil globules, absence of pyrenoids, peculiarity of attachment, and peculiarities of reproduction, at variance with what have been accepted up to this time as generic characters of Characium, it does not seem advisable to make a new genus for this form, without further study of living material.

Reinsch [3] has described a minute alga, *Dactylococcus Hookeri*, which he found growing attached to the small crustacean, *Cyclops bicaudatus*, collected in the neighborhood of Erlangen, Germany, in 1872. In 1874 he found another form, *Dactylococcus De Baryanus*,[3] growing on the same crustacean, *Cyclops bicaudatus*, collected in the same region. In the summer of 1877 he found the latter form, *Dactylococcus De Baryanus*, on "a somewhat smaller species" of *Cyclops* from pools a few miles west of the southern end of Lake Michigan. Again in the following summer, 1878, he discovered *Dactylococcus De Baryanus*, on *Cyclops bicaudatus* and on a species of *Lepidurus*,[4] taken from the water mains of the city of Boston. As Mr. F. S. Collins regards both of these species of Dactylococcus as belonging to the genus Characium, it is of interest to note what Reinsch [5] has described for the reproduction of *Dactylococcus De Baryanus*, the species which he found here in the vicinity of Boston. He describes as the earliest stage of development a slow-moving, green amoeboid cell of about 25 μ diameter, with red stigma. When these cells are elongated they have at the anterior hyaline end a single vibratile flagellum terminated by a bead-like thickening. After a short time these cells lose their power of movement, attach themselves to the surface of the

[1] Oltmanns, Dr. Fr., Morphologie und Biologie der Algen, Jena, 1904, Bd. 1, p. 175, quoting.

[2] Reinhardt, L., Entwickelungsgeschichte der Characien. Protok. d. Sekt.-Sitz., d. 5. Vers. Russ. Naturf. u. Arzte in Warschau, 1876. Jahresber. 4, p. 50.

[3] Reinsch, P. F., Contributiones ad Algologeam et Fungologeam, p. 78, pl. 11.

[4] In referring to the crustaceans found in the water mains of the city of Boston, Reinsch says, "Am 20 Juni waren die meisten untersuchten Thierchen (*Cyclops bicaudatus* und einer *Lepidurus*-species, von der deutschen verschieden) mit Parasiten besetzt." Inasmuch as Lepidurus is not known to occur east of the Great Plains, it is impossible to say what crustacean is referred to here.

[5] Reinsch, P. F., Beobachtungen über entophyte und entozoische Pflanzenparasiten, Botanische Zeitung, 1879, p. 38, pl. 1, figs. 21–24.

crustacean, and quickly develop into the adult Dactylococcus, the protoplast of which finally becomes divided into three or more daughter cells. The subsequent history of these daughter cells was not observed. Whether these features which have been described for *Dactylococcus De Baryanus* offer any solution for the gaps which at present I am unable to bridge in the life history of *Characium gracilipes* and *Characium cylindricum,* cannot be told until further observations can be made on living material of the two species of Characium and, if possible, on *Dactylococcus De Baryanus* itself.

Characium gracilipes and *Characium cylindricum* are hosts of a fungus, two stages of which are figured. Fig. 3 (pl. 79) shows an early stage; a late stage is represented in fig. 2 (pl. 79). The fungi occur attached to any part of either species, except the rhizoids and the distal region of the terminal hair in *Characium gracilipes,* and the region of attachment in *Characium cylindricum.* Presence of the fungus usually produces considerable modification in the shape of the Characium. In the earlier stages of the development of the fungus, the protoplast of the host shows a slight disturbance which increases as the development of the fungus advances. By the time the fungus has reached maturity, the protoplast of the host has usually quite disappeared, fig. 2 (pl. 79). This fungus will be the subject of a later paper.

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EXPLANATION OF PLATE 79.

All figures × 600.

Fig. 1. *Characium cylindricum,* cell containing 8 spores; e, spore escaping from lateral aperture; n, nucleus of spore; p, hyaline papilla at anterior end of spore.

Fig. 2. *Characium cylindricum,* empty cell with mature fungus sporangium (s) attached near distal end.

Fig. 3. *Characium gracilipes,* unicellular stage, with fungus cell (f) attached on side opposite the pyrenoid.

Fig. 4. *Characium gracilipes,* unicellular stage, a typical specimen; py, pyrenoid on convex side of cell; r, rhizoids.

Fig. 5. *Characium gracilipes,* 8 cells, distal and basal cells divided transversely; middle cells divided longitudinally.

Fig. 6. *Characium gracilipes,* 16 cells dividing to 32 cells by transverse division; all pyrenoids on convex side of cells; a typical condition.

Fig. 7. *Characium cylindricum,* cell containing 32 spores which are apparently motile.

Fig. 8. *Characium cylindricum*, cell developed into sporangium containing many small spores.
Fig. 9. *Characium gracilipes*, distal (x) and basal (z) ends of cell containing 64 spores; distal and basal cells divided transversely; other cells divided longitudinally.
Figs. 10, 11, 12 and 13. *Characium cylindricum*, unicellular stage; n, nucleus; ol, oil globules; two parietal chromatophores.
Fig. 14. *Characium gracilipes*, base of stipe, 2 rhizoids on one side.
Fig. 15. *Characium gracilipes*, base of stipe, two rhizoids, one of which is hooked.
Fig. 16. *Characium gracilipes*, base of stipe, two rhizoids dependent like pitch-fork.
Fig. 17. *Characium gracilipes*, base of stipe, 2 rhizoids, both hooked.
Fig. 18. *Characium gracilipes*, 2 rhizoids with numerous short branches.
Fig. 19. *Characium gracilipes*, 3 rhizoids, an unusual type.
Fig. 20. *Characium gracilipes*, 2 rhizoids, one shorter than the other.
Fig. 21. *Characium gracilipes*, 2 rhizoids with slight roughenings.
Fig. 22. *Characium gracilipes*, 2 rhizoids, the longest observed.
Fig. 23. *Characium cylindricum*, perforated distal end of cell containing spores; h, aperture.
Fig. 24. *Characium cylindricum*, cell containing 8 protoplasts, a typical specimen; n, nucleus.
Fig. 25. *Characium cylindricum*, a, base of cell attached to under side of main shaft of hair; c, base of cell attached to upper surface of main shaft of hair; b, base of cell attached to three of the smaller hairs.

Bartonia.— In hearty sympathy with every effort to give scholarly record to local floras we welcome the appearance of another American periodical, devoted as it appears chiefly to questions of taxonomy and plant-distribution. The new Bartonia, happily named and like most other Bartonias an annual, is edited by Mr. Stewardson Brown, who with Messrs. Joseph Crawford and Witmer Stone forms the Publication Committee of the Philadelphia Botanical Club. Its aims are to record in abstract the proceedings of the club and print short articles relating to the flora of the region about Philadelphia. The issue at hand is an admirably printed and completely indexed imperial octavo of 32 pages. In addition to introductory matter, the proceedings, history, and membership-list of the club, it contains the following articles: Botanical Trips to Northampton Co., Pa., by S. S. Van Pelt; Some Sand Dune Plants from Longport, N. J., by Joseph Crawford; and The Coastal Strip of New Jersey and the Rediscovery of Lilaeopsis, by Witmer Stone. Bartonia, dealing as it does with a flora closely related to that of our southwestern limits, will assuredly prove suggestive and interesting to botanists of New England.— B. L. R.